

COMPARATIVE STUDY OF EXOTIC TICK SPECIES FOR HUMAN REPELLENT REGISTRATION

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Abstract Repellent sprays are one of the most important tools for personal protection against ticks that transmit diseases to humans. There are detailed requirements for the registration of anti-tick products in Europe, e.g. when tested on humans, the tick must leave the treated skin within ≤ 1 minute or move a distance < 3 cm within a 3-minute test period. However, there are large differences between the species and, in particular, adult ticks are not repelled as effectively as the nymphs. If a product is claimed for use in tropical areas, *Hyalomma marginatum* (vector of e.g. Crimean-Congo haemorrhagic fever virus) or *Amblyomma variegatum* should also be tested according to EU guidelines (*A. americanum* for registration in North America). Against these two tick species, we tested the repellency of three active ingredients (A.I.) (DEET, Icaridin and Eukalyptus citriodora oil) commonly used in repellents against arthropod vectors worldwide. 20% ethanolic solutions of the A.I. were tested on either a temperature-controlled surface (in vitro) or on human forearms (in vivo). The results show that a walking distance of 3 cm is not sufficient as a repellent criterion. Adult ticks can cover distances of over 10 cm of the treated surface in a short time, before dropping off. This is consistent with the literature and suggests that fast-running ticks may be more difficult to repel than other tick species. The criterion of walking distance should be discussed, otherwise it can be that products will not meet the European guidance and enter the market.

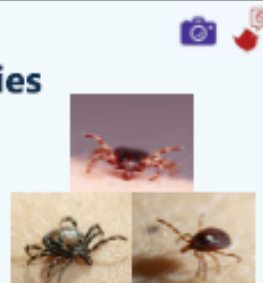
Key words ticks; repellent efficacy



Comparative study of exotic tick species for human repellent registration



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BACKGROUND

Repellent sprays are important tools to protect against ticks, vectors of diseases for humans. Product authorization in the EU is highly regulated and requires efficacy testing with e.g. *Hyalomma marginatum* (vector of e.g. Crimean-Congo haemorrhagic fever virus) or *Amblyomma variegatum* if claimed for use in tropical areas (*A. americanum* for registration in North America). There are also detailed requirements for testing repellents. When tested on humans, the tick must leave the treated skin within ≤ 1 minute or move a distance < 3 cm within a 3-minute test period in order to be effective^[1]. However, there are large differences concerning the walking speed between species and we speculate that fast running species may not be repelled by a repellent barrier only 3 cm wide.

RESULTS

In the plate assay, the repellents showed only minor effects on *H. marginatum* with Icaridin achieving the highest repellency (16.7 %). All repellents were more effective against both *Amblyomma* species. In a comparison of the three repellents, the natural-based component Citriodiol was the least effective.

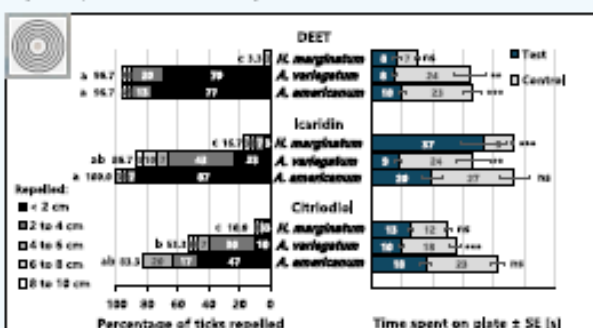


Fig. 1: Left: Effect of different repellents on walking distance until drop off (repellency) of *H. marginatum*, *A. variegatum* and *A. americanum* in the vertical plate assay. Right: Time the ticks spent on the plate surface (mean \pm SE) in control and test runs. Different letters indicate significant differences (Fisher's exact tests, two sided, Hochberg corrected, $P < 0.05$) on the numbers of repelled and not repelled ticks. U-Tests on the tick walking time in the corresponding test and control treatments. ***: $P < 0.001$, **: $P < 0.01$ not significant. $N=30$.

Tested on human skin, DEET was effective against all tick species tested. Using the > 3 cm criterion for "not repelled", efficacy off all repellents would have been significantly lower against most tick species tested.

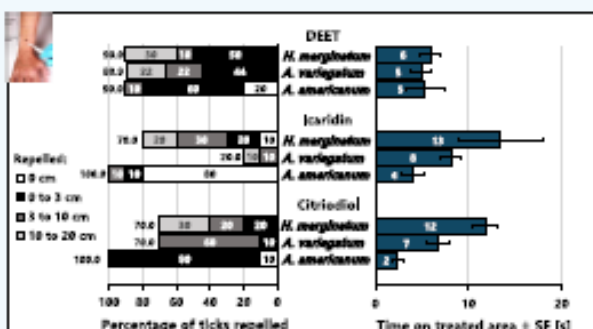


Fig. 2: Left: Tick walking distance on the treated arm area until the end of a test run (repelled: falling off, walking back; not repelled: walking > 20 cm). Right: Time the ticks spent on the test arm (mean \pm SE) until end of a test run. $N=9-10$.

CONCLUSIONS

The results show that a repellent barrier of 3 cm (both, on filter paper and on human skin) is insufficient to repel certain large tick species. Adult ticks can cover distances of over 10 cm on a treated surface in a short time before dropping off. This is consistent with the literature and suggests that fast-running ticks may be more difficult to repel than other tick species.

The criterion of walking distance should be discussed for fast-running species like *H. marginatum*. Otherwise, effective products may still not comply with the European guidance and can not enter the market.

MATERIALS & METHODS

Vertical plate assay

We screened common active ingredients (a.i.) of tick repellents for their efficacy against *H. marginatum*, *A. variegatum* and *A. americanum* using 20 % ethanolic solutions (w/w) on filter paper attached to a temperature-controlled plate.

Parameters:

Concentration of a.i.: 267 $\mu\text{g}/\text{cm}^2$

Surface temperature: 35 ± 0.5 °C

Test time: 3 min

Determination of walking distance until repellence behaviour (2 cm steps)

Ticks walking > 10 cm in any direction within 3 min were regarded as not repelled.

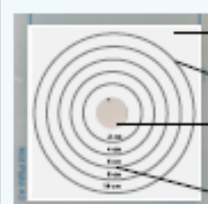


Fig. 3: Vertical plate assay setup. Adult ticks are individually released on the central untreated surface surrounded by filter paper treated with repellent. Tick behaviour is observed for a maximum of 3 min. Control runs were performed with solvent.

Arm assay

All active ingredients were tested *in vivo* on the human arm.

As tested tick species lack a pronounced negative geotactic response, ticks were individually guided upwards the treated arm over a maximum distance of 20 cm.

Parameters:

Concentration of a.i.: 267 $\mu\text{g}/\text{cm}^2$

Test time: 3 min

Determination of walking distance on treated area (3=10 cm steps).

Ticks that did not cross the treated area or crawled onto the treated area but turned back or fell off were regarded as repelled.

Ticks walking > 20 cm within 3 min were regarded as not repelled.



Fig. 4: Illustration of a marked forearm for testing with a *H. marginatum* adult. Lines from bottom to top: release line of ticks 3 cm below the repellent border; boundary line between treated and untreated area; boundary line 3 cm into the treated area (10 and 20 cm marks not shown). The upward movement of ticks was monitored for a maximum of 3 min. Test ticks were prescreened for sufficient activity on the untreated arm.

REFERENCE

[1] European Chemicals Agency (2023). Guidance on the Biocidal Products Regulation, Volume I: Efficacy assessment and evaluation, Parts B & C, Version 6.0, August 2023.

